IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In application of:
Jun-ichi Kawakami et al.: Examiner: Robert, Shiao
Serial No. 10/019,094: Group Art Unit: 1626
Filed on December 21, 2001:
For: PRODUCTION METHOD OF IMIDAZOLE DERIVATIVES:

DECLARATION UNDER 37 CFR 1,132

Honorable Commissioner of
Patents and Trademarks,
Washington, D.C. 20231

Sir:

I, Masami KUSAKA, a citizen of Japan, residing at 4-102-301, Gakuenhigashimachi 1-chome, Nishi-ku, Kobe, Japan sincerely declare:

That I was born on August 16, 1958 in Tokushima, Japan and graduated from Tokushima University in March 1987;

That I have been employed by Takeda Chemical Industries, Ltd., Japan since April 1987 and have been engaged in research work in the field of pharmacology and physiology on angiogenesis, endocrinological disorder and cancer;

That I was awarded Ph. D. degree from Tokushima University, Japan in 1989 on a doctoral thesis entitled "Possible induction of fatty acid cyclooxygenase in mouse osteoblastic cells (MC3T3-E1) by CAMP";

That I am a Research Scientist of Pharmaceutical Research Laboratories I, Pharmaceutical Research Division of the said company;

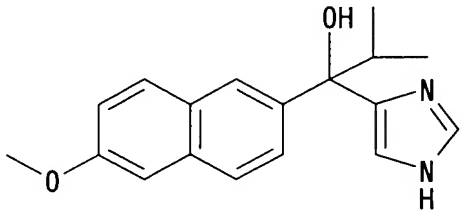
That I am a member of The Japanese Cancer Association and The Japan Endocrine Society; and

That I directed and supervised the following experiment to establish the utility of the compound of claim 17 of the present invention over the compound of Iwasaki et al. (Helv. Chim. Acta. 1976;59(8):2738-52) by comparing the inhibitory activity on steroid $C_{17,20}$-lyase by the compounds obtained using each compound as an intermediate, the results of which follow hereunder:

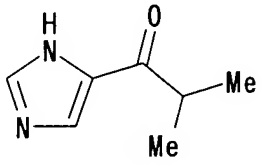
EXPERIMENT

Test compounds

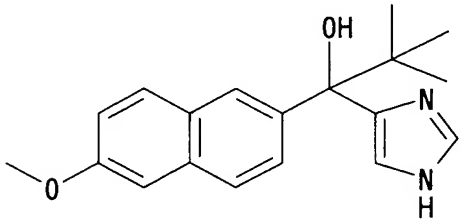
i) Compound A:



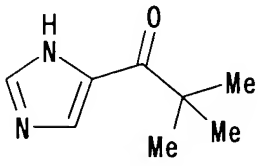
obtained using the following compound of claim 17 of the present invention as an intermediate.



ii) Compound B:



obtained using the following compound of Iwasaki et al. as an intermediate.



Method: Assay of inhibitory activity on a rat steroid $C_{17,20}$-lyase in vitro

Inhibitory activity was determined according to the method described in The Prostate, vol. 26, 140-150(1995) with some modifications.

Testes excised from 13-week-old male SD rats were homogenized, and testicular microsomes were prepared by a series of centrifugation. The microsome protein (7 μg/10 μl) was added to 10 μl of 100 mM phosphate buffer (pH 7.4) in which 10 nM (final concentration) [1,2-^{3}H]-17-α-hydroxyprogesterone, NADPH, and test compounds had been dissolved. The reaction mixture was incubated for 7 min at 37°C, the reaction was terminated by the addition of 40 μl of ethyl acetate, and the mixture was briefly centrifuged. The substrate and the products (testosterone and androstenedione) in the upper phase were separated by silica gel thin layer chromatography. Detection of the spots and measurement of the radioactivity were performed by a BAS 2000 Bioimage analyzer. The concentration of the test compounds necessary to reduce the concentration of the products by 50% (IC_{50}; the concentration of the control group in which no test compound was added was taken as 100%) was calculated.

The results are shown in Table 1.

[Table 1]

	Inhibitory activity on lyase (IC_{50} nM)
Compound A	33
Compound B	160

As is evident from the above data, compound A showed about 3 to 5 times stronger inhibitory activity on lyase than the compound B. Therefore, the compound of claim 17 is useful as compared to the compound of Iwasaki et al.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Osaka, Japan, this 29th day of August, 2003

.....Masami Kusaka.....

Masami KUSAKA